Size and Shape Differences in Genitalia of Males from Sympatric and Reproductively Isolated Populations of *Anthocoris antevolens* White (Heteroptera: Anthocoridae) in the Yakima Valley, Washington

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Ann. Entomol. Soc. Am. 98(4): 527–535 (2005)

ABSTRACT *Anthocoris antevolens* White (Heteroptera: Anthocoridae) is a widespread predatory bug in North America commonly associated with deciduous trees and shrubs. Unpublished observations showed that there is considerable geographic variation in male genitalia in this species and that the variation may lead to reproductive isolation among geographically separated populations. We show that male bugs from two sympatric populations in the Yakima Valley, Washington, one occurring on oak (*Quercus garryana* Douglas) and the other collected from willow (*Salix* sp.), differed in size and shape of the phallus and clasper. Mating trials showed that males from the oak source successfully inseminated females from the oak source in 75% of pairings; insemination success for males from the willow source paired with females from the willow source was somewhat lower at 62%. In nonlike crosses (oak × willow, willow × oak), males failed to inseminate the female in 100% of pairings, despite vigorous mating attempts by the males. Copulation duration was independent of population source. However, males from the willow source initiated copulation attempts significantly sooner in the assay than males from the oak source, irrespective of female source. We interrupted copulating pairs by freezing them with liquid nitrogen and showed that males in nonlike crosses generally had failed to fully inflate the phallus in the female. Results support statements made elsewhere that *A. antevolens* is actually composed of an unknown number of reproductively isolated cryptic species.

KEY WORDS Insecta, sexual selection, genitalia, morphometrics, mating isolation

PREDATORY BUGS IN THE FAMILY Anthocoridae are important sources of biological control in agricultural, forest, and stored products systems throughout the world. The genus *Anthocoris* Fallén includes ≈70 species distributed primarily in north temperate areas (Péricart 1972, 1996; Lattin 2000). Predators in this genus associate largely with trees and shrubs (Anderson 1962, Kelton 1978, Horton and Lewis 2000), and some species are of particular importance as sources of biological control in deciduous fruit tree crops (Westigard et al. 1968, McMullen 1971, Herard 1986).

A catalog to the Anthocoridae of North America lists 12 species of *Anthocoris* occurring north of Mexico (Henry 1988). The most widespread of these 12 species is *Anthocoris antevolens* White, which is found throughout Canada, south at least into the New England states in the east, north into Alaska, and south at least into Baja California and Arizona in the west (Van Duzee 1914, Anderson 1962, Kelton 1978, Lewis et al. 2005). The insect occurs on a number of tree and shrub species, especially species of *Salix* L. (Salicaceae), *Pyrus* L. (Rosaceae), *Populus* L. (Salicaceae), *Alnus* P. Miller (Betulaceae), and *Quercus* L. (Fagaceae) (Anderson 1962, Kelton 1978, Horton and Lewis 2000, Horton et al. 2004). The predator can be particularly abundant in pear orchards where it is an important natural enemy of pear psylla, *Cacopsylla pyricola* (Förster) (Homoptera: Psyllidae) (Westigard et al. 1968, Horton and Lewis 2000).

Clasper shape, phallus length, and phallus shape in males vary substantially among species of North American *Anthocoris* (Hill 1957; Kelton 1978; T.M.L., unpublished data). Indeed, taxonomic treatments of *Anthocoris* include claspers as diagnostic traits with which to confirm species' identifications (Hill 1957, Péricart 1972, Kelton 1978). Recently, we discovered that phallus length varies among geographically separated populations of *A. antevolens*, including among populations that are reproductively isolated (unpublished data). In this study, we compare the genitalia of males between populations of *A. antevolens* collected from willow (*Salix* sp.) and Oregon white oak (*Quercus garryana* Douglas) growing near Yakima, WA. Using both field-collected and laboratory-reared specimens, we compared males from the two populations in length of the phallus and clasper size and shape. We conducted mating experiments to determine whether

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males from one population could successfully inseminate females from the other population. We also contrasted certain mating behaviors between bugs from the two sources. Copulation duration was compared between the two populations of insects. Also, observations suggested that males from the willow source more rapidly initiated mating efforts than males from the oak source, and we explicitly tested this hypothesis here. Our overall objectives are to provide more evidence that *A. antevolens* is actually composed of an unknown number of reproductively isolated cryptic species that differ in size and shape of the male genitalia, and as shown previously (Horton et al. 2005), in aspects of sexual behavior.

Materials and Methods

Source of Insects. Adults and large nymphs of *A. antevolens* were collected in July and early August 2001 from *Q. garryana* and *Salix* sp. growing just west of the pear- and apple-growing regions of west Tieton, Yakima County, Washington. The oak specimens were collected over a 400-m distance along French Canyon Road in French's Canyon, at the western end of Tieton. The willow specimens were collected from a stand of *Salix* sp. growing at the entrance to French's Canyon (intersection of French Canyon Road and Noye Road), ≈3.2 km from the eastern edge of the oak stand. The collections included 95 bugs from oak and ≈120 bugs from willow.

A random sample of males was taken from both field collections and frozen at −80°C for determination of phallus length ($n = 26$ from willow and 23 from oak). The remaining insects were placed in ventilated plastic cages containing pear seedlings that had been infested with pear psylla. Rearing was done in environmental chambers at 23°C and a photoperiod of 16:8 (L:D) h, which allows continuous reproduction in *A. antevolens* (Horton et al. 1998). Once oviposition had commenced, 15 females were randomly collected from each population culture and placed individually in ventilated plastic cages (135 ml) containing psylla-infested pear seedlings. The bugs readily oviposited into the seedlings. Nymphs obtained from these field-collected females were then reared on pear seedlings and psylla in groups of 50–100 in ventilated plastic cages. The cages and bugs were kept at 23°C and a photoperiod of 16:8 (L:D) h. As the nymphs approached the final molt, they were moved individually to petri dishes lined with filter paper. Fresh, psylla-infested pear leaves were added to each dish every day. We recorded date of eclosion of each adult. These adults were used in the mating trials, for determination of phallus length (to supplement the same data obtained on field-collected bugs), and for analysis of clasper shape.

Phallus Length and Clasper Shape. Males of *Anthocoris* spp. inseminate females by transferring sperm into a membranous pouch located at the distal end of the female's copulatory tube. The copulatory tube arises as an invagination of the intersegmental membrane opening between segments 7 and 8 on the ventral surface of the female (Carayon 1953, Péricart 1972). Two structures of interest in males of *Anthocoris* with respect to the mating act are the left clasper and the phallus. During mating, the sclerotized left clasper is inserted partially into the female's copulatory tube. The male then inflates his membranous phallus, which is channeled initially into the female along a groove on the clasper (Carayon 1972, Péricart 1972). The fully inflated phallus is a double-walled tube, whose inner wall connects to the ejaculatory bulb and forms the duct through which seminal materials are moved (Fig. 1A, broken lines). The outer wall of the phallus (Fig. 1A, solid lines) is anchored at the genital capsule. In *A. antevolens*, a membranous pointed lobe of unknown function is present on the phallus just at the base of the inflated organ near the opening of the genital capsule. As the phallus deflates, the inner wall is retracted into the genital capsule, and the outer wall (to which the inner wall connects) trails the inner wall into the capsule. Thus, within the genital capsule, the phallus is stored as a single-walled organ (Fig. 1B). During storage, the outer wall is turned "outside-in," in contrast to what is seen in the inflated phallus. The mem-

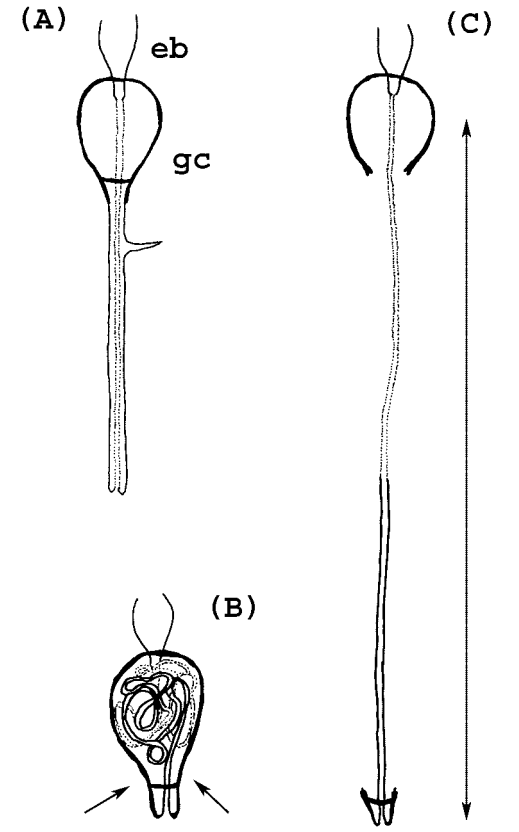

Fig. 1. (A) Fully inflated phallus. (B) Stored phallus within the genital capsule (arrows showing points of dissection). (C) Phallus pulled from genital capsule for measuring; measurement taken from mid-point of genital capsule to end of phallus (measurement depicted by arrow). eb, ejaculatory bulb; gc, genital capsule.

branous lobe, which is easily seen on the inflated organ, is thus on the inside of the single-walled tube in the stored, uninflated organ.

We were unable to develop a method for artificially inflating the phallus that consistently and unambiguously led to a fully inflated organ, so phallus length was determined by dissecting the uninflated organ from the genital capsule and measuring length of the dissected organ (Fig. 1C). The terminal segment of the male's abdomen was removed and placed in a small drop of Ringer's saline on a glass slide. The genital capsule was teased out under a dissecting microscope. The end of the capsule was detached (at arrows in Fig. 1B) by using #1 insect pins. A small drop of glycerin was added to the saline to reduce breakage and drying of the organ. The detached capsule end was then pulled across a thin layer of this solution until the phallus was straightened between its attachment points at each end of the broken capsule (Fig. 1C). The phallus was pulled taut enough that it was straightened but not stretched. The phallus would then remain in position while we measured its length (Fig. 1C, arrow), which was done using an ocular micrometer. One person did all dissections and measurements. Both field-collected and laboratory-reared first generation bugs were dissected. Mean length of the dissected phallus was compared between populations using a two-tailed *t*-test.

For 10 males from each population, we obtained two measurements of each phallus to provide an estimate of repeatability, which is a quantitative estimate of the importance of measurement error relative to true inter-specimen differences. The initial measurement was obtained for each phallus as described above. Each organ was then placed in two drops of glycerin on a coded microscope slide, where it remained for 20 min to 24 h (depending upon time constraints) until it could be remeasured. Slides kept overnight were refrigerated. A second measurement was then obtained by moving the organ onto a new drop of saline, adding a small drop of glycerin, and pulling the phallus across the glass slide until it was straightened but not stretched, as described above. Its length was then again estimated using an ocular micrometer. The second measurement was taken without knowing the original measurement and without knowing the source (oak versus willow) of the male. Repeatability was estimated following Lessells and Boag (1987). We felt obliged to estimate repeatability to ensure that we were consistent among bugs in how we straightened the organ on the slide before it was measured.

Clasper size and shape were described for an additional 15 randomly selected males from each population by using laboratory-reared offspring of the field-collected parental generation. The clasper of each male was removed from his abdomen by using microdissection tools and placed on a bed of glycerin borate jelly upon a microscope slide (Fig. 2). A drop of glycerin was added to make a shallow well in the jelly, in which the clasper was to be manipulated. Each slide was then placed beneath a DMLS compound microscope (Leica, Bannockburn, IL) equipped with a Spot Insight color digital camera (Diagnostic Instruments, Sterling Heights, MI). The clasper was manipulated until it was judged to be horizontal (perimeter of clasper in focus, neck of clasper slightly out of focus). The organ was then photographed. One person did all of the mounting of slides and took the photographs. The photograph was transferred onto a computer, and the image was then digitized using the tpsDig program (Rohlf 2001). We began each digitization at the tip of the hook and digitized in the same direction for each specimen (Fig. 2). Each digitization resulted in 95–120 points arranged approximately evenly along the perimeter of the clasper (Fig. 2). At the neck of the clasper, we attempted to digitize along an imaginary line describing the shortest distance between the two sides of the neck (Fig. 2). This location on a given clasper was somewhat arbitrary, in that its precise location seemed to depend upon positioning of the clasper on the slide (more precisely, in how horizontal the clasper was on the slide). One person digitized all of the images.

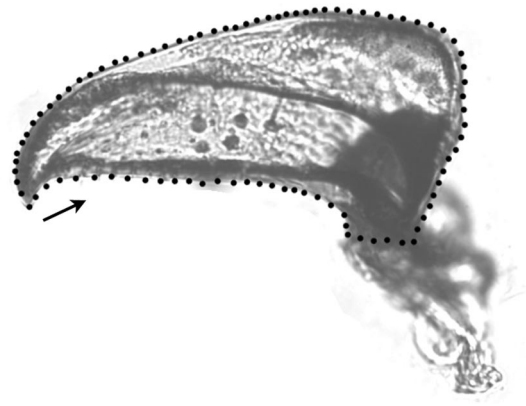

Fig. 2. Digitized clasper of a male from the willow source. Arrow shows direction of digitization.

Clasper shape was described quantitatively using elliptic Fourier analysis (EFA). This approach is useful for describing shapes of objects not easily measured using landmark-based methods (Liu et al. 1996, McLellan and Endler 1998, Arnqvist and Danielsson 1999) and has been used to describe outlines of genitalic structures in insects (Liu et al. 1996, Arnqvist and Danielsson 1999, Monti et al. 2001). We used the EFA program in Rohlf and Ferson (1992) to compute Fourier coefficients from the X-Y coordinates obtained for each outline (Fig. 2). Analyses were made invariant to clasper orientation, clasper rotation, and choice of starting point, by using options available in the EFA program. Visual examination of reconstructed outlines indicated that the first 20 harmonics for the Fourier analysis successfully described clasper shape; thus, analyses summarized below are based upon the use of 20 harmonics. A set of four coefficients accompanies each harmonic, leading to a set of 80 coefficients for each clasper.

Two analyses were done for each digitized clasper. First, Fourier coefficients were computed without standardization for clasper size (Rohlf and Ferson 1992), thus coefficients contained both size and shape information. Second, clasper size effects were removed by calculating a new set of coefficients, standardized using options provided in the EFA program; the second analysis thus is restricted to shape components. For both analyses, the matrix of Fourier coefficients was reduced into a smaller set of descriptors by using principal components analysis (PCA) (Ferson et al. 1985), with $n = 30$ (15 willow + 15 oak males). Scatter plots of scores from the first and second principal components were made to illustrate population separation. We used analysis of variance (ANOVA) on the scores from the first two principal components to determine whether clasper size and shape differed statistically between oak and willow bugs.

Mating Trials. Virgin female and male insects of 2–5 d in age were crossed in mating trials to determine percentage of matings leading to successful insemination. Offspring of field-collected bugs were used in the assays. Methods follow those used in previous studies (Horton et al. 2000, 2002). Assays were done in 6-cm-diameter plastic petri dishes at 22–24°C under fluorescent lighting. Females were placed individually in the petri dishes and allowed to settle for 15 min. A male from the same or other population was then added to each dish. All four possible crosses were tested. Sample sizes were 30 pairs per cross. Each pair was allowed 30 min to initiate copulation. If no mating occurred within 30 min, the assay was recorded as "no attempt." For males that attempted to mate with the female, we also recorded the amount of time that elapsed between the beginning of the assay and the male's initial attempt at achieving intromission. Copulation duration (defined as the interval of time between intromission and disengagement; Horton et al. 2000, 2002) also was recorded. The assay ended either after 30 min (no attempt) or after a mating pair had voluntarily separated. Females were dissected immediately after each assay to determine whether there was sperm in the sperm pouch.

A second set of pairings was done to determine whether nonlike crosses (i.e., oak × willow and willow × oak) were unsuccessful due to lack of success by the male in fully inflating his phallus. Again, 30 matings per cross were done for all four possible crosses. Pairs were placed in petri dishes and allowed to mate, as described above. Once the male had successfully inserted his clasper and settled on top of the female, we allowed 3 min of uninterrupted copulation to occur. At the end of the 3-min interval, we poured liquid nitrogen on the mating pair to flash-freeze the bugs. The male and female were then separated by carefully pulling the pair apart, taking care not to exert pressure on the male's abdomen. Length of the male's inflated or partially inflated phallus was then determined using an ocular micrometer. Phallus lengths were compared between type of cross by using two-tailed *t*-tests. Particularly for oak male × willow female crosses, males often failed to settle for an uninterrupted 3-min interval (evidenced by repeated insertion and removal of the clasper). The assays were discontinued at 30 min in those instances in which the male failed to completely settle for a 3-min interval.

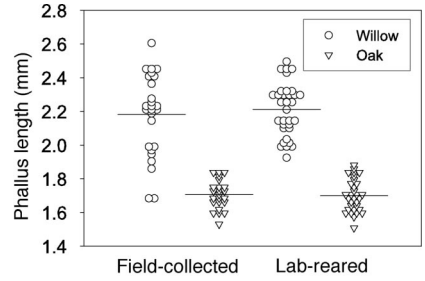

Fig. 3. Length of the dissected phallus in field-collected and laboratory-reared *A. antevolens* from two sources; n = 23–33 males per cluster of points. Field-collected individuals include some field-collected late instars that finished development in the laboratory on a pear psylla diet; laboratory-reared insects are offspring of field-collected adults reared from hatch to adult eclosion on pear psylla. Horizontal lines depict mean lengths.

Voucher Specimens. Voucher specimens of oak and willow forms have been deposited in the M.T. James Museum, Department of Entomology, Washington State University, Pullman.

Results

Phallus length differed between oak- and willow-collected bugs and between their respective laboratory-reared offspring (Fig. 3; 2 by 2 [population source x generation] ANOVA: population source (oak versus willow), $F_{1,\ 109} = 270.4$; $P < 0.001$). There was no difference in phallus length between field-collected and laboratory-reared bugs for either population source (Fig. 3; bug generation, $F_{1,\ 109} = 0.17$; $P = 0.68$ [population source × generation, $P > 0.25$]), suggesting that the difference in size of the phallus between oak- and willow-collected bugs in the parental insects was not caused by bugs from the two sources having developed on different diets. That is, the source effects noted for the field-collected adults (which had presumably developed on two different diets) also were observed in the laboratory-reared offspring (which had been fed a common diet of pear psylla). Little overlap in phallus length occurred between bugs from the two plant sources (Fig. 3). Variation in phallus length was larger in the willow form than in the oak form (Fig. 3); we have since discovered that the population of *A. antevolens* inhabiting *Salix* in the study area is actually composed of two reproductively isolated forms (Horton et al. 2005), which may explain the variation noted here.

Phallus length measurements were highly repeatable (Fig. 4). There was no statistical difference between mean phallus length estimated by the first measurement and second measurement (Fig. 4; paired *t*-test, $t = 0.9$, $P = 0.39$, $n = 20$). Repeatability estimates

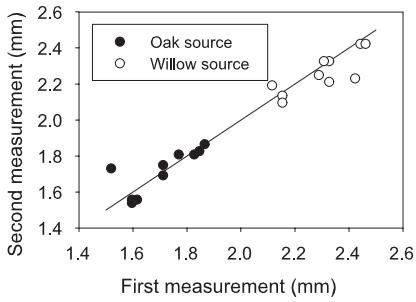

Fig. 4. Correlation between first and second measurements of the same dissected phallus for *A. antevolens* from two sources. Diagonal line depicts equivalence.

were 80.5 and 77.7% for the willow and oak specimens, respectively.

Principal components analysis of Fourier coefficients from the digitization of claspers led to separation of males from the oak versus willow sources along the first principal component (Fig. 5). Separation seemed to be better if the analysis was conducted using both size and shape information (Fig. 5A) than if done on shape components (Fig. 5B). The first and second principal components accounted for 80.5 and 12.2%, respectively, of the variation in size and shape (Fig. 5A), and for 70.1 and 13.0%, respectively, for the variation in shape (Fig. 5B). Outlines of claspers reconstructed from the Fourier coefficients (Rohlf and Ferson 1992) are shown for a male from willow (dotted outline in Fig. 5) and a male from oak (solid outline in Fig. 5), chosen to show separation along the first principal component in Fig. 5 (arrows depict location of the two males in the scatter plots). Claspers for males collected from willow were longer and (after size adjustment) narrower than those for males collected from oak (Fig. 5). ANOVA indicated that PC1 scores differed between oak and willow males in both scatter plots (Fig. 5A; $F_{1,\,28} = 125.8$, $P < 0.001$; Fig. 5B, $F_{1,\,28} = 27.0$, $P < 0.001$). There were no differences between oak and willow sources in the PC2 scores ($P > 0.20$ for both analyses).

In the mating trials, 57–87% of males attempted to mate the female, the lowest percentages occurring in the willow female × oak male (57%) and oak female × willow male (73%) crosses (Table 1). No females in the oak × willow or willow × oak crosses were inseminated despite mating attempts by males (Table 1). For the oak × oak and willow × willow crosses, 75% (18/24) and 62% (16/26), respectively, of males that attempted to mate inseminated the female (Table 1). In pairings that resulted in insemination, copulation duration was the same in oak × oak and willow × willow crosses (oak, $\bar{x} = 14.0 \pm 1.4$ min; willow, $\bar{x} = 11.1 \pm 1.4$ min; $t_{31} = 1.5$, $P = 0.16$). In assays in which the male attempted to mate the female, males from the willow source were more rapid in initiating the mating

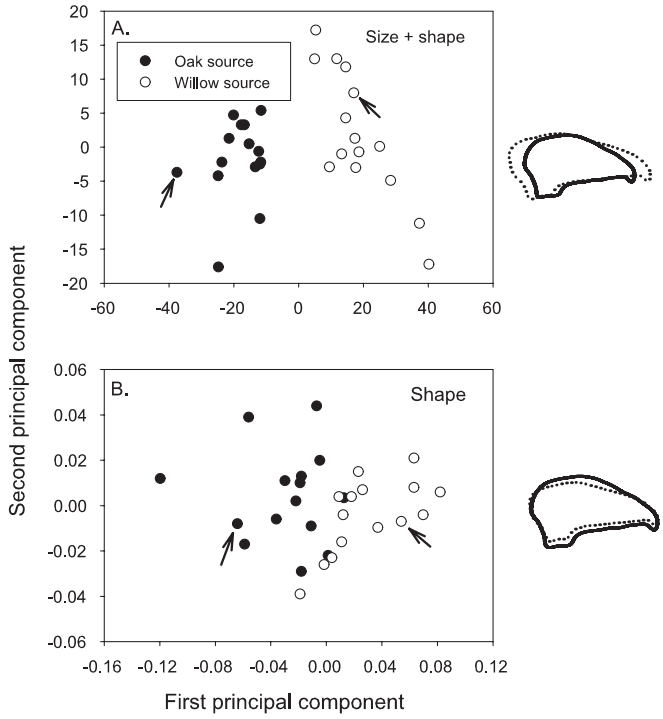

Fig. 5. Scatter plot for first and second principal components obtained from PCA of Fourier coefficients estimated from digitization of claspers. (A) Variation because of shape and size. (B) Variation because of shape. Outlines to right of scatter plots depict claspers reproduced from Fourier coefficients for a male from willow (dotted outline) and a male from oak (solid outline); arrows in scatter plot designate the two males.

Table 1. Frequency of mating success (sperm present), lack of success (attempted mating but no insemination), and lack of attempts for male and female *A. antevolens* collected originally from willow or from oak

Female source	Male source					
	Oak			Willow		
	Sperm present	Male attempts mating: no sperm	No attempt by male	Sperm present	Male attempts mating: no sperm	No attempt by male
Oak	18	6	6	0	22	8
Willow	0	17	13	16	10	4

Thirty pairs were monitored per type of cross. First generation offspring of field-collected insects were used in the assays. Each assay was 30 min.

attempt than males from the oak source, irrespective of female source (Fig. 6; male source, $F_{1, 85} = 12.2$, $P = 0.001$ [female source and the interaction both nonsignificant; $P > 0.25$] by ANOVA).

Lack of insemination success in the nonlike crosses apparently was due at least partially to males in those crosses being unable to fully inflate the phallus (Fig. 7). Figure 8 shows phallus lengths of males that were frozen during copulation 3 min after intromission and settling of the male on the female. Sample sizes are low for oak male × willow female crosses because of low numbers of males attempting to mate, and, for those that did attempt to mate, a low incidence of settling. In crosses involving willow-collected males, mean phallus length was larger for the willow × willow cross than the willow male × oak female cross (Fig. 8, right; $t_{28} = 3.4$, $P = 0.002$). No statistical test was done for crosses involving the oak males (Fig. 8, left), because of small numbers for the oak × willow cross.

Discussion

The oak- and willow-collected forms of *A. antevolens* both exhibited the typical phallus of *A. antevolens* (Fig. 1), which is distinct in appearance from those of all other North American species of *Anthocoris* except *Anthocoris musculus* (Say) and *Anthocoris dimorphicus* Kelton & Anderson (T.M.L., unpublished data). Claspers from both populations are also typical for *A. antevolens*, differing again in appearance from claspers of all other North American species, except *A. musculus* and *A. dimorphicus* (Kelton 1978). Both the oak- and willow-collected forms keyed readily to *A. antevolens* in both Hill (1957) and Kelton (1978), bugs from both sources having the typical *A. antevolens* external appearance: hemelytra entirely shiny; antennae not entirely black; and, pubescence moderately long and erect (Kelton 1978). Copulation duration, which can differ substantially among *Anthocoris* species (Horton et al. 2002), was the same in both forms. Last, males from both sources attempted to mate with females from the other source (Table 1).

Despite these similarities, the oak and willow forms did differ statistically in other characteristics. The dissected phallus of the willow form, although similar in shape to that in the oak form, was ≈0.5 mm longer than that in the oak form. Also, despite having the more-or-less typical clasper for *A. antevolens*, there were both size and shape differences in claspers of the two populations. The clasper of males from the oak source was shorter (Fig. 5A) but somewhat broader (Fig. 5B) than that in males from the willow source. Last, there were also behavioral differences in males from the two sources, in that males from the willow source were significantly quicker than males from the oak source in attempting to mount the female for copulation (Fig. 6).

The oak and willow forms seem to be reproductively isolated, despite attempts by males to mate with females of the other source (Table 1). We assume that differences in male genitalia between the oak and willow forms led to difficulties for the male in inseminating the female. Thus, males in nonlike crosses often failed to fully inflate the phallus (Figs. 7–8). Whether this absence of mating success was because of a lack of mechanical fit between males and females is not known, because we have been unable to develop an objective means for measuring the copulatory tube of the female. An alternative explanation is that males from nonlike sources failed to provide the correct cues to the female, and the female was able somehow to prevent the male from fully inflating the phallus. Eberhard (1996) (pp. 210–211) and Tallamy et al. (2002) discussed this issue for other species of insects, suggesting that females in some species may use internal

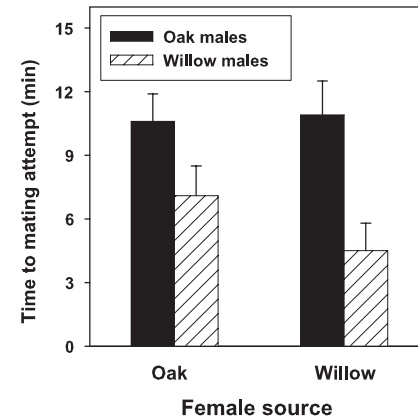

Fig. 6. Bar graph showing mean (±1 SE) number of minutes between onset of assay and initial attempt by male to mount female for oak-derived and willow-derived males mated with oak- or willow-derived females. Excludes males that failed to attempt mating during the 30-min assay; see Table 1 for sample sizes.

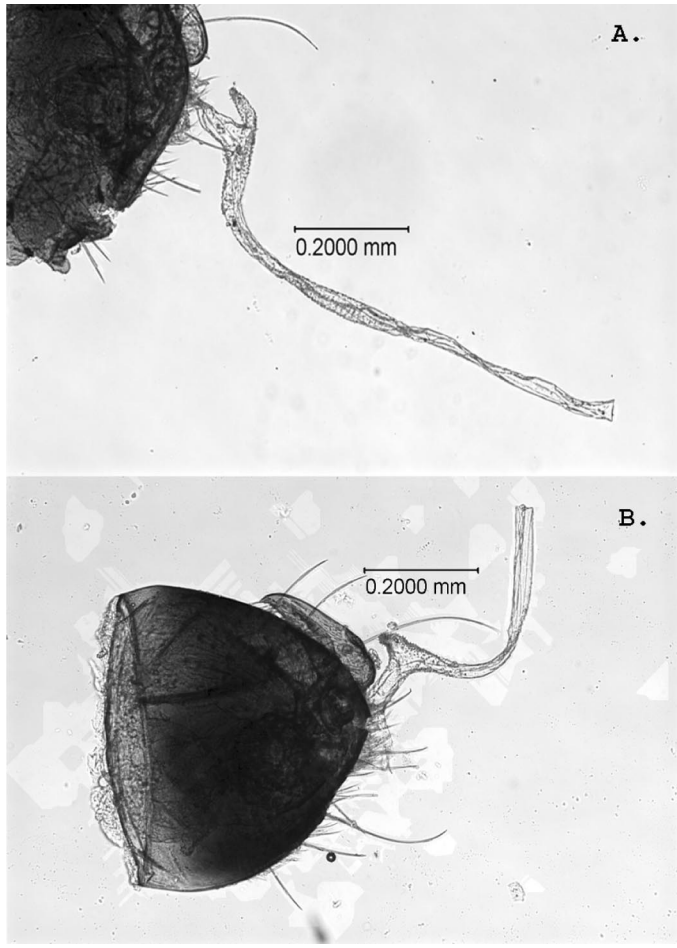

Fig. 7. Males from willow source interrupted in copulation showing fully inflated phallus observed in like crosses (A) and partially inflated phallus observed in nonlike crosses (B). The partially inflated phallus from the nonlike pairing in B is ≈50% in length of what is typically seen in a like cross. Note clasper readily visible in B at base of abdomen.

musculature to prevent males from inflating the phallus. Also, it is not yet clear what the consequences are of the differences in clasper size and shape between males of the two populations. It is possible that the clasper provides physical cues to the female that she uses to determine whether copulation should be allowed. Or, there may be some as yet undetermined lack of mechanical fit associated with having a clasper of the wrong size or shape that affects the male's ability to inseminate the female.

The morphological and behavioral data reported here suggest that *A. antevolens* in the Yakima valley is composed of more than a single species. The strongest support for this statement is the results of the mating trials, in which males from one plant source were 100% unsuccessful at inseminating females from the other plant source. Associated with this behavioral support is the demonstration that males from the two plant sources differed in size and shape of the phallus and clasper. For certain sibling species' groups, differences in male genitalia may be a primary morphological means for separating species, as shown in tephritid fruit flies (Iwahashi 1999a,b), drosophilid fruit flies (Coyne 1983), and certain Lepidoptera (Nice and Shapiro 1999). Increasing evidence supports the idea that sexual selection is responsible for strongly divergent variation in shape or size of male genitalia (Eber-

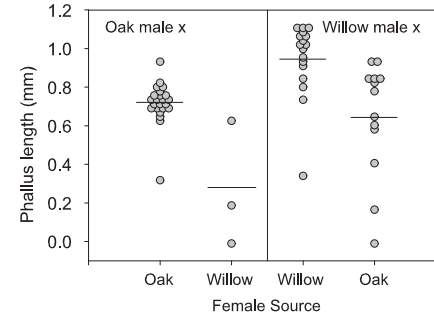

Fig. 8. Lengths of the inflated or partially inflated phallus for males in interrupted copulations between like- and nonlike crosses. Horizontal lines depict mean lengths.

hard 1996). Moreover, there is increasing acceptance that sexual selection can lead to reproductive incompatibility between populations, perhaps even under geographic sympatry (Via 2001). Whether sexual selection acting on male genitalia explains the reproductive incompatibility of these sympatric forms of *A. antevolens* is unknown.

Accurate taxonomic characterization of natural enemies underpins successful biological control programs (Rosen and DeBach 1973). As with other biological control organisms inhabiting pear orchards of the Pacific Northwest, *A. antevolens* seems often to colonize orchards from native habitats and host plants occurring adjacent to the orchards (Anderson 1962, Shimizu 1967, Horton and Lewis 2000). We showed previously that *Salix* and *Quercus* are important host plants for *A. antevolens* in habitats adjacent to the fruit-growing regions of the Yakima valley (Horton and Lewis 2000), but failed in that study to recognize that populations inhabiting oak differed anatomically and behaviorally from populations on willow. One practical consequence of this observation is that we may have been premature in inferring that all native plant species that support large numbers of *A. antevolens* in fruit-growing regions are likely to be important sources of the predator moving into orchards. That is, because we have not examined large numbers of pear-collected *A. antevolens* for genitalic morphology, we do not know whether bugs that we find in orchards are equally likely to have originated from oak or willow in the area. Our limited examinations of *A. antevolens* inhabiting pear orchards in western Yakima seem to indicate that the oak form is uncommon in pear orchards (unpublished data), although we caution that this observation is highly preliminary and merits a much closer look.

Acknowledgments

We thank Deb Broers and Toni Hinojosa for assistance. Earlier drafts of the article were reviewed by Carl Schaefer, Jim Hansen, and Peter Landolt. This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (#2002-35316-12266) to D.H. and T. Unruh, and by the Winter Pear Control Committee.

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Received 31 August 2004; accepted 24 March 2005.